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Multifunctionality of Sown Grassland Is Enhanced by Combining Four Complementary Species

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Multifunctionality of sown grassland is enhanced by combining four complementary species

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Abstract
We investigated species diversity effects and multifunctionality in an intensively managed grassland. A diversity experiment was set up with monocultures and mixtures comprising *Lolium perenne*, *Dactylis glomerata*, *Trifolium pratense*, and *Trifolium repens*, and was maintained for three years at 150 kg·N·ha^{-1}·year^{-1}. Ten functions were measured that represented i) forage production (aboveground biomass yield (μ), standard deviation of yield (σ), temporal stability (μ/σ), weed biomass), ii) N cycling (symbiotic-N_2-fixation, N efficiency, NO_3 in soil solution), and forage quality (crude protein content (CP), organic matter digestibility (OMD), metabolizable energy (ME)). We applied multivariate linear mixed-effects regression to estimate simultaneously species identity and diversity effects on the ten functions, and used the mean log response ratio (MLRR) across all functions to evaluate the diversity-multifunctionality relationship.

Across the three years, all functions regarding production and N cycling revealed significant beneficial effects in the four-species equi-proportional mixture (used as a reference) compared to averaged monocultures. The reference mixture had 61% more biomass yield, 8% less variation, 68% higher stability, 81% less weed biomass, 96% and 46% higher symbiotic-N_2-fixation and N-efficiency, respectively, and 87% less NO_3 (each $P \leq 0.05$, except variation). The reference mixture and averaged monocultures did not significantly differ in CP, OMD (g·kg^{-1}·yield), and ME (MJ·kg^{-1}·yield). This, however, resulted in significant beneficial effects between 52% and 72% in all three forage quality functions on a hectare basis (kg or MJ·ha^{-1}·year^{-1}). On average across functions, the four-species reference mixture had 1.8 times the performance of averaged monocultures, indicating enhanced multifunctionality in mixtures.

The multivariate framework in combination with the MLRR as a measure of overall multifunctionality proved to be an effective tool for the evaluation of the diversity-multifunctionality relationship. We conclude that sown grass-legume mixtures at moderate N fertilization promote high multifunctionality and are a 'ready-to-use' option for sustainable intensification of agriculture.

Introduction
There is evidence that higher plant species diversity promotes many relevant key ecosystem functions in agroecosystems, such as nutrient provision and biomass production (Allan et al. 2013). Due to the many functions benefiting from an increase in the number of plant species, higher species diversity is also thought to enhance overall multifunctionality (Lefcheck et al. 2015). This positive effect of species diversity arises because different species can be relevant to maintain different functions, or functions under different conditions.

Intensively managed grasslands have high economic and agronomic importance (O'Mara 2012). Such grasslands naturally contain fewer plant species than nutrient poor, extensively managed grasslands because only few species can cope with the short defoliation intervals in intensively managed systems. Given these conditions, it remains open whether only small increases in species richness positively affect multifunctionality. Despite the importance of productive grasslands, their diversity-multifunctionality relationship has never been evaluated.

Here, we investigate effects of species diversity on multifunctionality in an intensively managed grassland. A multivariate modelling framework was applied to simultaneously test for species interactive effects on multiple functions and their relative importance on overall multifunctionality (Dooley et al. 2015). Moreover, we developed a novel approach to measure multifunctionality using a mean log response ratio (of higher diversity mixtures against monocultures), which overcomes the generally acknowledged limitations of current methods (Gamfeldt and Roger 2017). We primarily tested whether increasing species diversity from monocultures to mixtures with only four species enhances overall multifunctionality. To this aim, we also investigated whether species complementarity effects on individual functions resulted in beneficial mixture effects driving the overall diversity-multifunctionality relationship.

Methods and Study Site

We used a dataset from a grassland diversity experiment at Zürich-Reckenholz, Switzerland, in the Atlantic central climatic zone of Europe. Four key forage species for ruminant production were selected based on the factorial combination of their functional traits related to temporal establishment and N acquisition (non-fixing for grasses, N_2-fixing for legumes). The species were *Lolium perenne* L. (fast-establishing grass), *Dactylis glomerata* L. (temporally persistent grass), *Trifolium pratense* L. (fast-establishing legume), and *Trifolium repens* L. (temporally persistent legume). Species were sown as monocultures and mixtures at a wide range of species relative abundances, and stands were maintained for three years following establishment. All plots received phosphorus and potassium in non-limiting amounts, and nitrogen (N) fertilization was 150 kg N ha^{-1} $year^{-1}$. In total, 42 plots (3 m × 6 m) were established and arranged in a fully randomised design. Consult Nyfeler et al. (2009) for full details of the experimental design, establishment, and maintenance.

Ten functions were measured representing i) forage production: aboveground biomass yield, standard deviation of yield, temporal stability, weed biomass; ii) N cycling: symbiotic N_2 fixation, N efficiency, NO_3 in soil solution; and iii) forage quality: crude protein content, organic matter digestibility, metabolizable energy content (Table 1). The measurement of all functions followed standard practices and is described elsewhere (Lehman and Tilman 2000; Nyfeler 2009; Nyfeler et al. 2009; Nyfeler et al. 2011; Agroscope 2013).

We applied the multivariate modelling framework (Dooley et al. 2015) to estimate simultaneously species identity and diversity effects of the ten functions. Linear mixed-effects regression was used to estimate parameters for each of the ten functions and the four species in monocultures and mixtures. The model included parameters for interactions between the grass and legume species, interactions between the two grass species, and between the two legume species. Based on the estimated model parameters, we calculated the beneficial mixture effect (BME) for each of the ten functions with:

$$\mathrm{BME}_k\ (\%) = \frac{\hat{y}_{\mathrm{equi}\_k} - \hat{y}_{\mathrm{avemono}\_k}}{\hat{y}_{\mathrm{avemono}\_k}} \times 100$$

with $\hat{y}_{\mathrm{equi}\_k}$ being the predicted functional performance of function *k* at the four-species equi-proportional mixture (chosen as a reference) and $\hat{y}_{\mathrm{avemono}\_k}$ the predicted performance of the average of monocultures. The diversity-multifunctionality relationship was evaluated using a new measure, the mean log response ratio (MLRR), which is the average of the log response ratios of all functions at the four-species equi-proportional mixture compared to the average of monocultures. For functions, where minimal values were regarded as of positive benefit, their LRR was first multiplied by -1. As such, a significantly positive MLRR indicates a positive diversity-multifunctionality relationship. All analyses presented here refer to data averaged across the three experimental years.

Results

Beneficial species interactions in mixtures

We observed highly significant species interactions in mixtures that favoured the majority of functions. Beneficial interactions were strongest between grass and legume species (being complementary in N acquisition), and – although to a lesser extent – were also evident between the two grass species and between the two legume species (each pair being complementary in temporal establishment; no table shown).

The cumulative effect of all species interactions led to beneficial mixture effects for all forage production and N cycling functions (Table 1). For example, the four-species equi-proportional reference mixture had 61% more yield, 8% less variation (SD_{yield}), 68% higher stability, 81% less weed biomass, 96% more symbiotic N_2 fixation, 46% higher N efficiency, and 87% less NO_3 in the soil solution compared to averaged monocultures (each at least $P \leq 0.05$, except SD_{yield}). NO_3 in the soil solution of mixtures was negligibly low (< 0.1 mg NO_3-N $liter^{-1}$). For the three forage quality functions, there were next to no mixture benefits if functions were expressed as content (g kg^{-1} yield in CP and OMD, MJ kg^{-1} yield in ME, Table 1). Notably, this outcome appeared despite significantly higher yield in mixtures. As a result, CP, digestible OM, and ME per hectare (ha^{-1} $year^{-1}$) were much greater in mixtures than in averaged monocultures, with beneficial mixture effects being in the range of 52-72%.

Table 1: Percent beneficial mixture effects for ten functions representing forage production, N cycling, and forage quality, measured over three experimental years. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns: $P > 0.05$

Service	Function		Unit *(Ratio)*	Targeted direction	Beneficial mixture effect
Production	Aboveground biomass yield	Yield	Mg ha^{-1} $year^{-1}$	positive	61% ***
	Standard deviation of yield	SD_{yield}	Mg ha^{-1} $year^{-1}$	negative	-8% ns
	Temporal stability of yield	Stability	*Yield / SD_{yield}*	positive	68% **
	Aboveground weed biomass	Weed biomass	Mg ha^{-1} $year^{-1}$	negative	-81% ***
N cycling	Symbiotic N_2 fixation	N_{sym}	kg ha^{-1} $year^{-1}$	positive	96% ***
	N efficiency	N efficiency	*N yield / N applied*	positive	46% ***
	NO_3 in soil solution	NO_3	mg $liter^{-1}$	negative	-87% *
Forage quality	Crude protein content	CP	g kg^{-1} yield	positive	-8% ns
	Organic matter digestibility	OMD	g kg^{-1} yield	positive	-2% ns
	Metabolizable energy content	ME	MJ kg^{-1} yield	positive	-3% ns

Significantly enhanced multifunctionality in mixtures

Beneficial mixture effects were evident without trade-off between functions (Table 1). Due to this result, multifunctionality was significantly enhanced in mixtures, with the four-species equi-proportional mixture having on average 1.8 times the performance of averaged monocultures (Fig. 1).

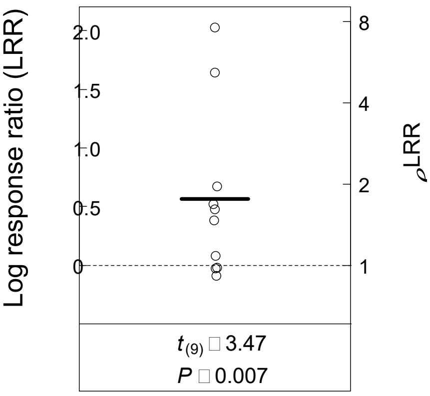

Figure 1: Enhanced multifunctionality in grass-legume mixtures expressed as the mean log response ratio (MLRR) (horizontal line) across ten functions (circles). The log response ratio was calculated for each function for a mixture with equal proportions of all four species compared to averaged monocultures, based on multivariate regression analysis. The inference (*t*- and *P*-value) refers to a test of the MLRR against zero. Circles are slightly scattered horizontally to improve their visibility.

Discussion

Our selected species were cultivars known to perform well in pure stands under intensively managed conditions. However, it was not clear how their combination in mixtures affects multifunctionality, as multifunctionality also depends on the species' interactions within a community. It is therefore surprising and of high practical importance that multifunctionality was enhanced by a factor of almost two by using only four species. We attribute this strong diversity effect to the targeted combination of species with complementary functional traits (Lüscher et al. 2011; Storkey et al. 2015).

In our grass-legume system, species complementarity in N acquisition was most important to result in beneficial mixture effects for many functions. Legume species are capable of symbiotic N_2 fixation, which allows them to cover their own N demand, while grass species rely solely on N uptake from soil and fertilizers. Moreover, growing these species in mixtures allows for transfer of symbiotically fixed legume N to the grasses (Høgh-Jensen and Schjoerring 1997; Nyfeler et al. 2011). Previous studies have shown that these processes substantially enhance biomass and protein yield (Nyfeler et al. 2009; Nyfeler et al. 2011; Suter et al. 2015), improve stability (Haughey et al. 2018) and enhance weed suppression (Suter et al. 2017; Connolly et al. 2018). In our experiment, positive interactions between legume and grass species also increased N efficiency and kept NO_3 in soil solution at low levels. Additional analyses revealed that complementarity in temporal establishment further improved yield and stability, and suppressed weeds, all of which are highly important for sustainable production.

Our new measure, the MLRR across functions, proved to be valuable in evaluating overall multifunctionality. The log response ratio is easy to apply and is widely used in ecology, probably because of its desirable statistical properties (Hedges et al. 1999). In our approach, the individual drivers of multifunctionality could still be identified through the multivariate modelling framework (Dooley et al. 2015), which is important to identify opposing behaviours between single functions and overall multifunctionality. Knowledge of both, the performance of individual functions and overall multifunctionality, is essential for appropriate decisions for management and stakeholders.

We have demonstrated that grass-legume mixtures increased yields with no decrease in forage quality, increased stability, maintained weed suppression, enhanced N efficiency but reduced N leaching to low levels, all of which increased multifunctionality. These features align well with recent demands to produce more with same resources and concomitantly preserve the environment (Godfray and Garnett 2014). The species of our experiment are used worldwide in production-oriented grasslands and their cultivation in mixtures provides a 'ready-to-use' approach. Given their many benefits and the lack of adverse effects on the environment, legume-based multispecies grasslands should become a key option for the sustainable intensification of agriculture.

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